DATA EVALUATION RECORD
ACUTE LC_{50} TEST WITH AN ESTUARINE/MARINE SHRIMP
§72-3(C)

Data Requirement:		
	PMRA Data Code	9.4.2
	EPA DP Barcode	DP349851
	OECD Data Point	IIA 8.11.1
	EPA MRID	47560303
	EPA Guideline	OPPTS 850.1035

1. **CHEMICAL:** Saflufenacil PC Code No.: 118203

2. **TEST MATERIAL:** BAS 800H Metabolite M07 Purity: 95.4%

3. **CITATION**

Authors:	T. Minderhout, T.Z. Kendall, H.O. Krueger and C. Holmes
Title:	BAS 800H Metabolite M07: A 96-Hour Static Acute Toxicity Test with the Saltwater Mysid (*Americamysis bahia*)
Study Completion Date:	October 8, 2008
Laboratory:	Wildlife International, Ltd., Easton, Maryland
Sponsor:	BASF Corporation, RTP, North Carolina
Laboratory Report ID:	147A-246
MRID No.:	475603-03
DP Barcode:	D349851

4. **REVIEWED BY:** John Marton, Staff Scientist, Cambridge Environmental, Inc.

Signature: **Date:** 11/24/08

APPROVED BY: Teri S. Myers, Senior Scientist, Cambridge Environmental, Inc.

Signature: **Date:** 12/01/08

5. **APPROVED BY:** Primary Reviewer: Anita Pease, Senior Biologist, U.S. EPA

Signature: **Date:** 06/09/09

6/9/09

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Secondary Reviewer: Ann Lee, HC-PMRA-EAD

Signature: **Date:** 06/09/09

Secondary Reviewer: Farzad Jahromi, DEWHA-APVMA

Signature: **Date:** 06/09/09

6. DISCLAIMER: This document provides guidance for EPA and PMRA reviewers on how to complete a data evaluation record after reviewing a scientific study concerning the acute toxicity of a pesticide to shrimp. It is not intended to prescribe conditions to any external party for conducting this study nor to establish absolute criteria regarding the assessment of whether the study is scientifically sound and whether the study satisfies any applicable data requirements. Reviewers are expected to review and to determine for each study, on a case-by-case basis, whether it is scientifically sound and provides sufficient information to satisfy applicable data requirements. Studies that fail to meet any of the conditions may be accepted, if appropriate; similarly, studies that meet all of the conditions may be rejected, if appropriate. In sum, the reviewer is to take into account the totality of factors related to the test methodology and results in determining the acceptability of the study.

7. EXECUTIVE SUMMARY:

In a 96-h acute toxicity study, saltwater mysid (*Americamysis bahia*) were exposed to BAS 800 H metabolite M07 at nominal concentrations of 0 (negative control), 6.3, 13, 25, 50, and 100 mg a.i./L under static conditions. Mean-measured concentrations were <2.0 (<LOQ, control), 6.2, 13, 25, 50, and 98 mg a.i./L. The 96-h LC_{50} was >98 mg a.i./L. Up to four lethargic mysids were noted in each of the 50 and 98 mg a.i./L treatment levels, and one mortality occurred at the 50 mg a.i./L treatment level. Therefore, the NOAEC was determined by visual interpretation of the mortality and sublethal effects data as 25 mg a.i./L. Based on the results of this study, the M07 metabolite of BAS 800 H is categorized as practically non-toxic to mysid shrimp on an acute toxicity basis in accordance with the classification system of the U.S. EPA.

This toxicity study is classified as **ACCEPTABLE** to the **U.S. EPA** and as **FULLY RELIABLE** to **PMRA and APVMA** as it is scientifically sound and satisfies the guideline requirement for an acute estuarine/marine invertebrate toxicity study.

Results Synopsis

LC_{50}: >98 mg ai/L 95% C.I.: N/A

NOAEC: 25 mg ai/L
Probit Slope: N/A

8. STUDY PARAMETERS

Age or Size of Test Organism:	Juveniles (<24 hours old)
Definitive Test Duration:	96-hours
Study Method:	Static
Type of Concentrations:	Mean measured

9. GUIDELINE DEVIATIONS: This study was conducted following guidelines outlined in the U.S. Environmental Protection Agency Series 850- Ecological Effects Test Guidelines, OPPTS Number 850.1035: *Mysid Acute Toxicity Test*; U.S. Environmental Protection Agency, Standard Evaluation Procedure, *Acute Toxicity Test for Estuarine and Marine Organisms*; and ASTM Standard E729-96: *Standard Guide for Conducting Acute Toxicity Test on Test Materials with Fishes, Macroinvertebrates and Amphibians*. The following deviation from OPPTS 850.1035 was noted:

The TOC concentration in the dilution water was not reported.

This deviation does not impact the acceptability of the study.

10. SUBMISSION PURPOSE: This study was submitted to provide effects on mysid (*Americamysis bahia*) following acute exposure to BAS 800H Metabolite M07 (Saflufenacil) for the purpose of new chemical registration.

11. MATERIALS AND METHODS

A. Test Organisms

Guideline Criteria	Reported Information
Species Preferred species are *Mysidopsis bahia*, *Penaeus setiferus*, *P. duorarun*, *P. aztecus* and *Palaemonetes sp.*	*Americamysis bahia*
Age Juvenile, mysids should be ≤24 hours old	Juveniles (<24 hours old)
Supplier	In-house cultures
All shrimp are from same source?	Yes
All shrimp are from the same year class?	Yes

B. Source/Acclimation

Guideline Criteria	Reported Information
Acclimation Period minimum 10 days	Continuous
Wild caught organisms were quarantined for 7 days?	N/A
Were there signs of disease or injury?	No
If treated for disease, was there no sign of the disease remaining during the 48 hours prior to testing?	N/A

Guideline Criteria	Reported Information
Feeding No feeding during the study and no feeding for 24 hour before the beginning of the test if organisms are over 0.5 g each. Mysids should be fed throughout the study.	Mysid cultures were fed live brine shrimp (*Artemia* sp.) nauplii (Brine Shrimp Direct, Odgen, Utah), occasionally enriched with ALGAMAC-200 (Aquafauna, Hawthorn, California) to prevent cannibalism. During the test, mysids were fed live brine shrimp (*Artemia* sp.) nauplii daily.
Pretest Mortality <3% mortality 48 hours prior to testing	None reported

C. Test System

Guideline Criteria	Reported Information
Source of dilution water Soft reconstituted water or water from a natural source, **not** dechlorinated tap water	Test water was collected at Indian River Inlet, Delaware and subsequently filtered and diluted to a salinity of ~20‰ with well water. The freshly-collected seawater was passed through a sand filter to remove particles greater than approximately 25 µm and pumped into a 37,800-L storage tank. The 20‰ diluted water was filtered to 0.45 µm and passed through a UV sterilizer to remove fine particles and microorganisms prior to its use in the test.
Does water support test animals without observable signs of stress?	Yes
Salinity 30-34 ‰ (parts per thousand) for marine (stenohaline) shrimp and 10-17 ‰ for estuarine (euryhaline) shrimp, weekly range < 6 ‰	20‰

Guideline Criteria	Reported Information
Water Temperature Approx. 22 ± 1 °C	24.6-26.3 °C
pH 8.0-8.3 for marine (stenohaline) shrimp, 7.7-8.0 for estuarine (euryhaline) shrimp, monthly range < 0.8	7.7-8.2
Dissolved Oxygen Static: ≥ 60% during 1st 48 hrs and ≥ 40% during 2nd 48 hrs, Flow-through: ≥ 60%	6.5 (96 hours) to 7.7 mg/L (≥89% saturation)
Total Organic Carbon Should be <5 mg/L in reconstituted seawater	Not Reported
Test Aquaria 1. Material: Glass or stainless steel 2. Size: 19.6 L is acceptable for organisms ≥ 0.5 g (e.g. pink shrimp, white shrimp, and brown shrimp), 3.9 L is acceptable for smaller organisms (e.g. mysids and grass shrimp). 3. Fill volume: 15 L is acceptable for organisms ≥ 0.5 g, 2-3 L is acceptable for smaller organisms.	Glass 2 L 1500 mL
Type of Dilution System Must provide reproducible supply of toxicant	N/A- test was conducted under static conditions
Flow Rate Consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period	N/A

Guideline Criteria	Reported Information
Biomass Loading Rate Static: ≤ 0.8 g/L at ≤ 17°C, ≤ 0.5 g/L at > 17°C; flow-through: ≤ 1 g/L/day (N/A for mysids)	N/A
Photoperiod 16 hours light, 8 hours dark	16L:8D with a 30-minute transition period of low-light intensity; light intensity at test initiation was 285 lux at the surface of the water of one representative test chamber.
Solvents Not to exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-through tests	Solvent: N/A- a solvent was not used Maximum conc.: N/A

D. Test Design

Guideline Criteria	Reported Information
Range Finding Test If LC_{50} >100 mg/L with 30 shrimp, then no definitive test is required.	A 96-hour non-GLP range-finding study was conducted by exposing ten mysids to nominal concentrations of 0 (negative control), 0.24, 0.81, 2.7, 9.0 and 30 mg ai/L. After 96- hours of exposure mortality was 10% in the control and 20, 30, 10, 30 and 10% in the nominal 0.24, 0.84, 2.7, 9.0 and 30 mg ai/L treatment groups, respectively.
Nominal Concentrations of Definitive Test Control & 5 treatment levels; a geometric series in which each concentration is at least 60% of the next higher one.	0 (negative control), 6.3, 13, 25, 50 and 100 mg ai/L
Number of Test Organisms Minimum 20/level, may be divided among containers	20 per level, equally divided among 2 replicates

Guideline Criteria	Reported Information
Test organisms randomly or impartially assigned to test vessels?	Yes
Biological observations made every 24 hours?	Yes
Water Parameter Measurements 1. Temperature Measured constantly or, if water baths are used, every 6 hrs, may not vary > 1EC 2. DO and pH Measured at beginning of test and ever 48 h in the high, medium, and low doses and in the control	1. Temperature was measured in each test vessel at test initiation and termination. Temperature was also continuously measured in a container of water placed adjacent to the test chambers. 2. DO and pH were measured in each test vessel at test initiation and every 24 hours thereafter.
Chemical Analysis needed if solutions were aerated, if chemical was volatile, insoluble, or known to absorb, if precipitate formed, if containers were not steel or glass, or if flow-through system was used	Samples were collected from the newly prepared batches of solution of each treatment and control group at test initiation and from each replicate test chamber of each treatment and control group at 96 hours. All samples were collected at mid-depth, placed in glass vials, and analyzed using HPLC using variable wavelength detection set at 220 nm.

12. REPORTED RESULTS

A. General Results

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Signed and dated No Data Confidentiality, GLP and Quality Assurance statements were provided. This study was conducted in compliance with the Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency (40 CFR Parts 160 and 792, 17 August 1989); OECD Principles of Good Laboratory Practice (ENV/MC/CHEM (98) 17); and Japan MAFF (11 NohSan, Notification No. 6283, Agricultural Production Bureau 1, October 1999), with the following exception: period analyses of well water for potential contaminants were not performed according to Good Laboratory Practice Standards, but were performed using a certified laboratory and standard U.S. EPA analytical methods.
Recovery of Chemical	Recoveries at test initiation ranged from 97.6 to 100% of nominal and recoveries at test termination ranged from 97.7 to 101% of nominal.
Control Mortality Not more than 10% of control organisms may die or show abnormal behavior.	0%
Raw data included?	Yes
Signs of toxicity (if any) were described?	Yes

Mortality

Concentration (mg ai/L)		Number of Shrimp	Cumulative Number Dead
Nominal	Mean Measured		Hour of Study

			24	48	72	96
Control	<2.00	20	0	0	0	0
6.3	6.2	20	0	0	0	0
13	13	20	0	0	0	0
25	25	20	0	0	0	0
50	50	20	0	0	0	1
100	98	20	0	0	0	0

Other Significant Results: The single mortality observed at the 50 mg ai/L treatment level was not considered to be treatment related due to the lack of mortality at the highest treatment level. The resulting LC_{50} value was estimated to be >98 mg ai/L.

Beginning at 24 hours and continuing throughout the duration of the definitive exposure period, 4 mysids in each of the two highest treatment groups were observed to be lethargic; no other sub-lethal effects were observed in the control or remaining treatment levels. Therefore, the study authors determined the NOAEC value to be 25 mg ai/L.

B. Statistical Results

Method: The lack of mortality precluded the statistical calculation of LC_{50} values; therefore, the LC_{50} value was estimated to be greater than the highest concentration tested. The NOAEC value was determined by visual interpretation of the mortality and observation data.

96-hr LC_{50}: >98 mg ai/L 95% C.I.: N/A
NOAEC: 25 mg ai/L
Probit Slope: N/A

13. VERIFICATION OF STATISTICAL RESULTS

Parameter	Result
Binomial Test LC_{50} (C.I.)	>98 mg ai/L
Moving Average Angle LC_{50} (95% C.I.)	>98 mg ai/L
Probit LC_{50} (95% C.I.)	>98 mg ai/L
Probit Slope	N/A
NOAEC	25 mg ai/L

14. REVIEWER'S COMMENTS:

The reviewer's results agreed with the study authors.

The results from the periodic screening analysis of the dilution water indicated the presence of the following elements: barium (0.0056 mg/L), bromide (64.5 mg/L), calcium (276 mg/L), chloride (12,000 mg/L), magnesium (763 mg/L), potassium (296 mg/L), sodium (6,490 mg/L) and sulfate (1,540 mg/L).

At test initiation and termination, all test and control solutions appeared clear and colorless in the test chambers.

The in-life portion of the definitive toxicity test was conducted from September 18 to 22, 2008.

15. REFERENCES:

U.S. Environmental Protection Agency. 1996. Series 850- Ecological Effects Test Guidelines (*draft*), OPPTS Number 850.1035: *Mysid Acute Toxicity Test.*

U.S. Environmental Protection Agency. 1985. Standard Evaluation Procedure: *Acute Toxicity Test for Estuarine and Marine Organisms (Shrimp 96-Hour Acute Toxicity Test).* Hazard Evaluation Division. Office of Pesticide Programs. EPA-540/9-85-010. Washington, DC.

ASTM Standard E729-96. 1996. *Standard Guide for Conducting Acute Toxicity Tests on Test*

Materials with Fishes, Macroinvertebrates and Amphibians. American Society for Testing and Materials.